# Circulating neuregulin1-beta in heart failure with preserved and reduced left ventricular ejection fraction

Hage, Camilla; Wardell, Eva; Linde, Cecilia; Donal, Erwan; Lam, Carolyn S. P.; Daubert, Claude; Lund, Lars H.; Mansson-Broberg, Agneta

*Published in:*
ESC Heart Failure

*DOI:*
10.1002/ehf2.12615



*Publication date:*
2020

ESC HEART FAILURE
*ESC Heart Failure* (2020)
Published online in Wiley Online Library (wileyonlinelibrary.com) **DOI:** 10.1002/ehf2.12615

ORIGINAL RESEARCH ARTICLE

# Circulating neuregulin1-β in heart failure with preserved and reduced left ventricular ejection fraction

Camilla Hage[1,3]*, Eva Wärdell[2,3], Cecilia Linde[1,3], Erwan Donal[4], Carolyn S.P. Lam[5,6,7], Claude Daubert[4], Lars H. Lund[1,3] and Agneta Månsson-Broberg[2,3]

[1]*Department of Medicine, Karolinska Institutet – Solna, Stockholm, Sweden;* [2]*Department of Medicine, Karolinska Institutet – Huddinge, Huddinge, Sweden;* [3]*Heart and Vascular Theme, Karolinska University Hospital, SE-171 76, Stockholm, Sweden;* [4]*Département de Cardiologie & CIC-IT U 804, Centre Hospitalier Universitaire de Rennes, Rennes, France;* [5]*National Heart Centre Singapore, Duke-National University of Singapore, Singapore, Singapore;* [6]*University Medical Centre Groningen, Groningen, The Netherlands;* [7]*The George Institute for Global Health, Sydney, Australia*

## Abstract

**Aims** Neuregulin1-β (NRG1-β) is released from microvascular endothelial cells in response to inflammation with compensatory cardioprotective effects. Circulating NRG1-β is elevated in heart failure (HF) with reduced ejection fraction (HFrEF) but not studied in HF with preserved EF (HFpEF).

**Methods and results** Circulating NRG1-β was quantified in 86 stable patients with HFpEF (EF ≥45% and N-terminal pro-brain natriuretic peptide >300 ng/L), in 86 patients with HFrEF prior to and after left ventricular assist device (LVAD) and/or heart transplantation (HTx) and in 21 healthy controls. Association between NRG1-β and the composite outcome of all-cause mortality/HF hospitalization in HFpEF and all-cause mortality/HTx/LVAD implantation in HFrEF with and without ischaemia assessed as macrovascular coronary artery disease was assessed. In HFpEF, median (25th–75th percentile) NRG1-β was 6.5 (2.1–11.3) ng/mL; in HFrEF, 3.6 (2.1–7.6) ng/mL ($P$ = 0.035); after LVAD, 1.7 (0.9–3.6) ng/mL; after HTx 2.1 (1.4–3.6) ng/mL (overall $P < 0.001$); and in controls, 29.0 (23.1–34.3) ng/mL ($P$ = 0.001). In HFrEF, higher NRG1-β was associated with worse outcomes (hazard ratio per log increase 1.45, 95% confidence interval 1.04–2.03, $P$ = 0.029), regardless of ischaemia. In HFpEF, the association of NRG1-β with outcomes was modified by ischaemia (log-rank $P$ = 0.020; $P_{interaction}$ = 0.553) such that only in ischaemic patients, higher NRG1-β was related to worse outcomes. In contrast, in patients without ischaemia, higher NRG1-β trended towards better outcomes (hazard ratio 0.71, 95% confidence interval 0.48–1.05, $P$ = 0.085).

**Conclusions** Neuregulin1-β was reduced in HFpEF and further reduced in HFrEF. The opposing relationships of NRG1-β with outcomes in non-ischaemic HFpEF compared with HFrEF and ischaemic HFpEF may indicate compensatory increases of cardioprotective NRG1-β from microvascular endothelial dysfunction in the former (non-ischaemic HFpEF), but this compensatory mechanism is overwhelmed by the presence of ischaemia in the latter (HFrEF and ischaemic HFpEF).

**Keywords** HFpEF; HFrEF; Neuregulin1-β; Coronary artery disease; Prognosis

*Received: 5 September 2019; Revised: 22 November 2019; Accepted: 23 December 2019*
**Correspondence to: Camilla Hage, Heart and Vascular Theme, Karolinska University Hospital, SE-171 76 Stockholm, Sweden. Tel: +46 8 517 700 00.*
*Email: camilla.hage@sll.se*
*Clinical Trial Registration: ClinicalTrials.gov NCT00774709 https://clinicaltrials.gov*

## Introduction

Heart failure (HF) is categorized according to left ventricular ejection fraction (LVEF) as being reduced (HFrEF) or preserved (HFpEF), and lately also a mid-range (HFmrEF)[1], all common and associated with poor prognosis. Pharmacological treatments in HFrEF have decreased morbidity and mortality, but prognosis remains poor. In HFpEF, often including patients with HFmrEF, trials have been neutral suggesting different or heterogeneous underlying pathophysiologies.

In both HFpEF and HFrEF, endothelial dysfunction is associated with incident HF, disease progression, and adverse

outcomes,[2] but its role may differ. In HFrEF, direct cardiomyocyte injury is a key trigger, while in HFpEF, co-morbidity-driven endothelial dysfunction plays a dominant role.[3]

Neuregulin-1 (NRG1) is a membrane bound vasculoactive peptide and part of the epidermal growth factor family. It is released by proteolytic cleavage from endothelial cells in the microvasculature in several tissue types, including the heart, in response to inflammation, ischaemic, and oxidative stress.[4–6] NRG1 affects cardiomyocytes by activating tyrosine kinase receptors, such as ERBB4 and ERBB2, activating downstream signalling through the phosphatidylinositol 3-kinase and mitogen-activated protein kinase pathways thereby inhibiting apoptosis and inducing cardiomyocyte proliferation.[5,6] NRG1 has at least 31 isoforms with different epidermal growth factor-like domains, but NRG1-β is the most extensively studied.

In the HFrEF myocardium, both the ERBB2 and ERBB4 receptors are downregulated, whereas NRG-1 expression is upregulated,[7] and circulating NRG1-β has been associated with HF severity and mortality.[8] In HFpEF, NRG1-β and its prognostic impact have not been studied. Furthermore, the implications of NRG1-β may differ depending on the HF aetiology (ischaemic versus non-ischaemic).[8]

We aimed to explore circulating NRG1-β, its prognostic role in HFpEF and HFrEF, and the impact of ischaemia in patients with HFrEF, before and after left ventricular assist device (LVAD) therapy, and heart transplantation (HTx).

# Methods

## Patients

Patients were recruited between 21 May 2007 and 29 December 2011 at Karolinska University Hospital, Stockholm, Sweden. Patients with decompensated HFpEF ($n$ = 86), N-terminal pro-brain natriuretic peptide (NT-proBNP) >300 ng/L, and LVEF ≥45% were enrolled. Follow-up at the hospital was performed in stable condition after 4–8 weeks including blood sampling and echocardiography (regarded as baseline in the present analysis). Patients with HFrEF ($n$ = 86) referred for assessment for LVAD or Tx and LVEF <40% were enrolled. Blood sampling was performed prior to LVAD/HTx ($n$ = 86), cross-sectionally 1 year after LVAD implantation ($n$ = 26) or HTx ($n$ = 35).

Healthy individuals ($n$ = 21) with systolic blood pressure < 150 mmHg, body mass index < 35, free from hypertensive treatment, and known macrovascular coronary artery disease (CAD) were blood-sampled.

## Laboratory analyses

Blood samples were collected in a fasting state in the morning in ethylenediaminetetraacetic acid tubes and centrifuged, and plasma was aliquoted and stored in −70 °C until analysis. NRG1-β was assessed with enzyme-linked immunosorbent assay RAB0388, Sigma-Aldrich Sweden AB. It includes antibody pre-coated plate(s) and other components needed to perform the assay. This NRG1-β solid-phase, sandwich enzyme-linked immunosorbent assay detects the amount of the specific protein bound between a matched antibody pair. After incubation periods and wash steps, a substrate solution was added that produces a measurable signal. The intensity of this signal is proportional to the concentration of target present in the original specimen. The intensity was measured with a Microplate Reader (SpectraMax 250, Molecular Devices, USA) at 450 nm.

N-terminal pro-brain natriuretic peptide was analysed by proBNPII (Roche Diagnostics, Bromma, Sweden). In addition to insulin-like growth factor (IGF)-1 values with age adjusted standard deviation scores calculated from the regression of the IGF-1 concentrations of healthy adult subjects (standard deviation score = ((10lnIFG-1 − observed + 0.00693*age) − 2.581)/0.120) were calculated. Insulin resistance was assessed according to homeostatic model assessment of insulin resistance calculated as ([glucose*Insulin]/22.5; with glucose in mmol/L and insulin in mU/L) and estimated glomerular filtration rate to the Modification of Diet in Renal Disease equation.

## Doppler–echocardiography

The echocardiographic assessment was performed on a ViVid 7 echo-platform (GE VingMed, Horten, Norway) and analysed in a dedicated core centre in Hôpital Pontchaillou-CHU, Rennes, France. Each examination was interpreted once, and measurements were performed three times and averaged by an echocardiographist (E. D.) blinded to the specific clinical history of the patient. Diastolic dysfunction was assessed as ratio of early transmittal velocity to mitral annular early velocity (E/e′) >15 and structural heart disease as either left atrial volume index (LAVI) calculated as left atrial volume in millilitres divided by body surface area in $m^2$) >34 mL/$m^2$ or left ventricular hypertrophy defined as left ventricular mass index ≥95 g/$m^2$ in women and ≥115 g/$m^2$ in men, respectively.[1]

## Endpoints

Patients with HFpEF were followed until 30 September 2012 when vital status was assessed by telephone contact or by

the Swedish National Patient Register and Population Register. The primary composite endpoint was defined as time to mortality from any cause or first hospitalization due to HF. All HF hospitalizations were adjudicated and defined according to clinical judgement by the local investigator, and additionally, centrality was validated to confirm the presence of HF at hospitalization.

In patients with HFrEF, implantation of LVAD or HTx was assessed by patient charts in December 2014 and vital status by the Swedish National Patient and Population Registers. In such patients, the primary composite endpoint was death from any cause, implantation of LVAD, or HTx.

### Statistics

Descriptive data in *Table 1* is expressed as median and quartiles (Q1;Q3) or number (%) and compared by the Wilcoxon rank-sum test and Fisher's exact test in HFpEF versus HFrEF and Kruskal–Wallis test and $\chi^2$ between HFrEF, LVAD, and HTx. Difference in NRG1-β concentrations between HF-groups and controls and NYHA classes was determined by ANOVA and analysis of covariance. Bivariate correlations with plasma or serum biomarkers and clinical variables were established by linear and logistic regression analyses.

Association between NT-proBNP and NRG1-β concentrations in HFpEF and HFrEF were tested with ischaemia defined as the presence of macrovascular CAD as an interaction term.

Associations with outcome were determined with Kaplan–Meier and Cox proportional hazards models with ischaemia as an interaction term. In the final multivariable Cox regression model, four clinically significant covariates, age, sex, estimated glomerular filtration rate, and NT-proBNP, were included. Due to non-normal distribution plasma and serum, biomarkers were analysed in log-transformed format. *P*-values were two-sided, and statistical significance was set at 0.05. Statistical analyses were performed using SAS software version 9.3 (SAS Institute, Cary N. C, USA).

### Ethics

The KaRen and MetAnEnd studies were conducted according to International Conference on Harmonization and Good Clinical Practice guidelines. The investigation conforms with the Declaration of Helsinki and was approved by the ethical review board at Karolinska Institutet. Written informed consent was obtained from all participants prior to study participation.

## Results

### Patients

Clinical characteristics of HFpEF, HFrEF, patients receiving LVAD or HTx, and healthy controls are presented in *Table 1*. Patients with HFpEF were median 73 (interquartile range 67;79) years old and 51% were women. NT-proBNP was 1000 [469;2330] ng/L, LVEF was 64% [58;68], E/e′ ratio was 10.8 [8.3;14.0], and LAVI 43.3 mL/m$^2$ [37.2;53.8]. A proportion of 23% had increased filling pressures E/e′ >15; 67% had e′ <9; 89% had enlarged left atria, LAVI >34 mL/m$^2$; and 61% fulfilled criteria for left ventricular hypertrophy. The median age of patients with HFrEF was 63 [52;68] years, and 19% were female. NT-proBNP concentration was 3290 ([1430;5860] ng/L and LVEF 21 [15;28] %; both *P*-values versus HFpEF <0.001). Macrovascular CAD was present in HFpEF and HFrEF in 29 [34%] vs. 38 (44%), respectively (ns).

### Neuregulin1-β concentrations

As depicted in *Figure 1* NRG1-β was higher in HFpEF, (6.5 [2.1-11.3]ng/mL compared to HFrEF 3.6 [2.1-7.6]ng/mL (*P* = 0.035). Concentrations decreased after LVAD treatment, 1.7 [0.9-3.6]ng/mL and HTx 2.1 [1.4-3.6]ng/mL (overall *P* < 0.001). NRG1-β was substantially lower in all groups, individually and overall, compared to controls: 29.0 ng/mL [23.1-34.3] (*P* = 0.001).

Neuregulin1-β did not correlate with age, sex, comorbidities, and echocardiographic measures in either HFpEF or HFrEF (*Table 2*). Among biomarkers in HFpEF, only haemoglobin correlated with NRG1-β (β = 0.25; *P* = 0.029), and in HFrEF, ST2 (β = 0.43; *P* < 0.001), adiponectin (β = 0.33; *P* = 0.004), and IGFBP1 (β = 0.37; *P* = 0.001).

In HFpEF, NRG1-β decreased with increasing NYHA class (overall *P* = 0.013) in contrast to HFrEF where NRG1-β was higher in NYHA class IV versus III (*P*-value = 0.030). NRG1-β stratified by NYHA class between HFpEF and HFrEF displayed no significant difference.

In ischaemic HFpEF, NRG1-β was insignificantly lower (5.2 [1.5–8.9] ng/mL) compared with non-ischaemic HFpEF (7.0 [2.3–11.9] ng/mL; *P* = 0.274), whereas there was no difference in ischaemic HFrEF (3.3 [2.1–6.3]) and non-ischaemic HFrEF (3.9 [2.1–7.7]); *P* = 0.484). Interestingly, stratifying the HFpEF group by ischaemia, there was a trending association between increasing NRG1-β and NT-proBNP in ischaemic but not in non-ischaemic HFpEF (*Figure 2A*; $P_{interaction}$ = 0.589). This pattern was not seen in HFrEF (*Figure 2B*).

**Table 1** Baseline characteristics in the 86 HFpEF, 86 HFrEF, 26 LVAD, 21 HTx patients, and 21 healthy controls

| Variable | HFpEF ($n$ = 86) | HFrEF ($n$ = 86) | $P$-value (HFrEF versus HFpEF) | LVAD ($n$ = 26) | HTx ($n$ = 35) | $P$-value (overall HFrEF) | Controls ($n$ = 21) | $P$-value (controls versus HFpEF) | $P$-value (controls versus HFrEF) |
|---|---|---|---|---|---|---|---|---|---|
| **Patient history** | | | | | | | | | |
| Age (years) | 73 (67;79) | 63 (52;68) | *<0.001* | 53 (45;66) | 51 (44;61) | *0.001* | 67 (61;70) | *<0.001* | *0.088* |
| Gender (females) | 44 (51%) | 16 (19%) | *<0.001* | 6 (23%) | 6 (17%) | *0.832* | 12 (57%) | *0.808* | *<0.001* |
| **Medical history** | | | | | | | | | |
| COPD | 17 (20%) | 10 (12%) | *0.208* | 1 (4%) | 1 (3%) | *0.189* | | | |
| T2DM | 28 (33%) | 25 (29%) | *0.741* | 6 (29%) | 6 (23%) | *0.710* | | | |
| Cancer | 15 (17%) | 8 (9%) | *0.178* | 2 (8%) | 1 (3%) | *0.474* | | | |
| Hypertension | 68 (79%) | — | — | — | — | — | | | |
| Atrial fibrillation /flutter | 52 (60%) | 45 (52%) | | 9 (35%) | 18 (51%) | *0.270* | | | |
| Coronary artery disease | 29 (34%) | 38 (44%) | *0.211* | 11 (42%) | 10 (29%) | *0.274* | | | |
| Whereof revascularized | 17 (59%) | 30 (79%) | *<0.001* | 9 (82%) | 9 (90%) | | | | |
| Revascularized | 17 (20%) | 33 (38%) | *0.011* | 12 (46%) | 11 (31%) | *0.050* | | | |
| NYHA class I | 19 (22%) | 1 (1%) | *<0.001* | 1 (4%) | 22 (63%) | *<0.001* | | | |
| NYHA class II | 47 (55%) | 4 (5%) | | 14 (54%) | 10 (29%) | | | | |
| NYHA class III | 20 (23%) | 67 (78%) | | 7 (27%) | 2 (6%) | | | | |
| NYHA class IV | 0 (0%) | 14 (16%) | | 1 (4%) | 1 (3%) | | | | |
| **Measurements** | | | | | | | | | |
| Weight (kg) | 83.5 (72;98) | 83.8 (72;94) | *0.951* | 80 (69;88) | 84 (77;98) | *0.310* | | | |
| BMI (kg/m$^2$) | 28.5 (25.0;32.9) | 27.3(23.2;30.1) | *0.016* | 25 (23;27) | 26 (23;30) | *0.351* | 25 (23;26) | *0.002* | *0.071* |
| Systolic blood pressure (mmHg) | 140 (130;150) | 105 (96;120) | *<0.001* | 103 (100;113) | 118 (95;130) | *0.495* | 125 (120;140) | *0.002* | *<0.001* |
| Diastolic blood pressure (mmHg) | 80 (70;85) | 70 (62;80) | *<0.001* | 70 (63;73) | 74 (65;83) | *0.485* | 80 (75;83) | *0.790* | *<0.001* |
| Heart rate (b.p.m.) | 70 (60;80) | 70 (60;74) | *0.484* | 70 (64;71) | 83 (74;88) | *0.001* | | | |
| **Treatment** | | | | | | | | | |
| ARB | 28 (33%) | 31 (36%) | *0.748* | 9 (35%) | 23 (37%) | *0.007* | | | |
| ACE inhibitor | 42 (49%) | 51 (59%) | *0.221* | 16 (62%) | 0 (0%) | *<0.001* | | | |
| Beta-blocker | 69 (80%) | 85 (99%) | *<0.001* | 23 (88%) | 15 (43%) | *<0.001* | | | |
| Thiazide diuretics | 14 (16%) | | | | | | | | |
| Potassium sparing diuretics | 18 (21%) | 58 (67%) | *<0.001* | 15 (58%) | 3 (9%) | *<0.001* | | | |
| Loop diuretics | 61 (71%) | 75 (87%) | *0.014* | 15 (58%) | 11 (31%) | *<0.001* | | | |
| Calcium channel blocker | 27 (31%) | 4 (5%) | *<0.001* | 5 (19%) | 9 (26%) | *0.003* | | | |
| Anticoagulants | 47 (55%) | 53 (62%) | *0.440* | 23 (88%) | 1 (3%) | *<0.001* | | | |
| Antiplatelet | 29 (34%) | 22 (26%) | *0.317* | 19 (73%) | 31 (89%) | *<0.001* | | | |
| Statins | 38 (44%) | 38 (44%) | *1.000* | 6 (23%) | 31 (41%) | *<0.001* | | | |
| **ECHO parameters** | | | | | | | | | |
| LVEF (%) | 64 (58; 68) | 21 (15;28) | *<0.001* | 20 (13;30) | 58 (55;63) | *<0.001* | | | |
| LVEDd (mm) | 47 (43;53) | 66 (61;75) | *<0.001* | 56 (52;66) | 46 (42;50) | *<0.001* | | | |
| LAVI (mL/m$^2$) | 44 (38; 52) | | | | | | | | |
| Left ventricular mass index (g/m$^2$) | 114 (95;143) | | | | | | | | |
| Men | 125 (102;157) | | | | | | | | |

(*Continues*)

**Table 1** (continued)

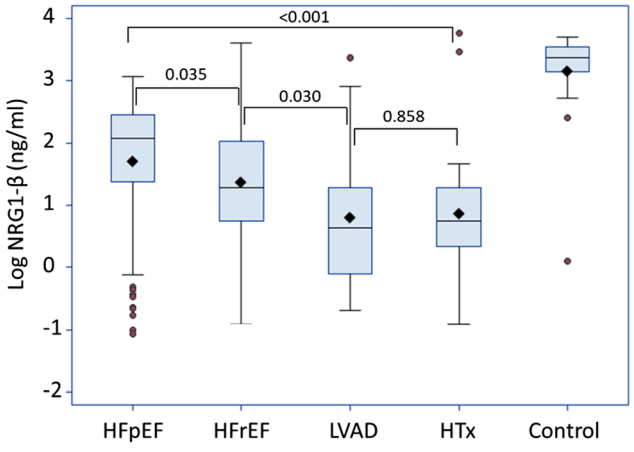
| Variable | HFpEF ($n$ = 86) | HFrEF ($n$ = 86) | $P$-value (HFrEF versus HFpEF) | LVAD ($n$ = 26) | HTx ($n$ = 35) | $P$-value (overall HFrEF) | Controls ($n$ = 21) | $P$-value (controls versus HFpEF) | $P$-value (controls versus HFrEF) |
|---|---|---|---|---|---|---|---|---|---|
| Women | 109 (94;136) | | | | | | | | |
| E/A ratio | 1.3 (0.9;2.1) | | | | | | | | |
| E/e' ratio | 10.8 (8.3;14.0) | | | | | | | | |
| E' | 8.0 (7.0;10.0) | | | | | | | | |
| IVRT (diastole) | 94 (77;113) | | | | | | | | |
| Mitral VTI | 23 (16;30) | | | | | | | | |
| E-wave deceleration time (ms) | 203 (156;228) | | | | | | | | |
| **Biochemistry** | | | | | | | | | |
| NT-proBNP (ng/L) | 1000 (469;2330) | 3290 (1430; 5860) | *<0.001* | 1115 (824;1760) | 247 (166;469) | <0.001 | 67 (31;110) | *<0.001* | *<0.001* |
| MR-proANP (pmol/L) | 313 (193;381) | 449 (326;593) | *<0.001* | 276 (224;301) | 118 (96;158) | <0.001 | | | |
| MR-proADM (nmol/L) | 1.22 (0.93; 1.62) | 1.33 (0.94; 1.95) | *0.215* | 0.92 (0.75;1.30) | 0.67 (0.56;0.86) | <0.001 | | | |
| Hb (g/dL) | 131 (122;142) | 133 (122;144) | *0.619* | 130 (111;138) | 129 (115;138) | 0.158 | | | |
| Creatinine (μmol/L) | 84 (73;107) | 113 (98;143) | *<0.001* | 104 (74;125) | 96 (83;112) | 0.004 | | | |
| eGFR (MDRD) (mL/min/1.73m$^2$) | 70 (54;85) | 59 (44;71) | *0.004* | 65 (55;83) | 77 (58;91) | <0.003 | | | |
| Copeptin (pmol/L) | 14 (9;21) | 28 (18;45) | *<0.001* | 16 (7;18) | 12 (5;19) | <0.001 | | | |
| Suppression of tumourigenicity 2 (ng/mL) | 23 (17;31) | 35 (23;51) | *<0.001* | 28 (26;32) | 22 (17;26) | <0.001 | | | |
| Sodium (mmol/L) | 141 (140;143) | 138 (136;140) | *<0.001* | 138 (136;139) | 140 (138;142) | <0.001 | | | |
| Potassium (mmol/L) | 3.9 (3.7;4.2) | 4.2 (3.9;4.6) | *<0.001* | 4.4 (4.0;4.7) | 3.9 (3.7;4.2) | 0.008 | | | |
| Glucose fasting (mmol/L) | 5.6 (5.1;7.5) | 5.5 (4.9;6.9) | *0.091* | 5.6 (5.0;6.2) | 5.3 (4.9;7.2) | 0.974 | | | |
| Insulin (μU/mL) | 11.2 (8.4;16.9) | 11.1 (6.7;16.7) | *0.642* | 11.7 (6.9;18.0) | 9.5(6.8;14.0) | 0.721 | | | |
| HOMA-IR | 3.4 (2.0;5.6) | 2.8 (1.1;5.1) | *0.034* | 2.5 (1.63;4.4) | 2.2 (1.5;3.1) | 0.899 | | | |
| Adiponectin (mg/L) | 11.7 (7.8;20.1) | 13.7 (7.0;21.1) | *0.471* | 10.5 (96;272) | 11.5 (6.8;15.9) | 0.210 | | | |
| Leptin (ng/L) | 24.1 (11.7;51.7) | 15.0 (6.2;33.2) | *0.015* | 10.6 (7.9;36.6) | 10.4 (7.0;18.2) | 0.531 | | | |
| IGF1 (mikrog/L) | 174 (137;206) | 149 (105;219) | *0.101* | 138 (96;272) | 214 (170;306) | 0.001 | | | |
| SD score IGF1 | 1.22 (0.62;1.93) | 0.09 (−1.40;1.62) | *<0.001* | −0.14 (−1.4;1.1) | 1.0 (−0.4;2.1) | 0.053 | | | |
| IGFBP1 (mikrog/L) | 48 (28;78) | 65 (29;101) | 0.074 | 54 (35;77) | 28 (20;39) | <0.001 | | | |

ACE, angiotensin converting enzyme; ARB, angiotensin II receptor blocker; BMI, body mass index; COPD, chronic obstructive pulmonary disease; eGFR, estimated glomerular filtration rate; Hb, haemoglobin; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; HOMA-IR, homeostatic model assessment of insulin resistance; HTx, heart transplantation; IGF1, insulin-like growth factor 1; IVRT, isovolumic relaxation time; LAVI, left atrial volume index; LVAD, left ventricular assist device; LVEDd, left ventricular end diastolic diameter; LVEF, left ventricular ejection fraction; MDRD, Modification of Diet in Renal Disease; MR-proADM, mid-regional pro-adrenomedullin; MR-proANP, mid-regional pro atrial natriuretic peptide; NT-proBNP, N-terminal pro-brain natriuretic peptide; NYHA, New York Heart Association; SD, standard deviation; T2DM, type 2 diabetes mellitus; VTI, velocity-time integral.

Continuous variables are presented as median and lower and upper quartiles (Q1;Q3) and categorical variables as numbers ($n$) and percentages.


DOI: 10.1002/ehf2.12615

**Figure 1** Concentrations of log NRG1-β in HFpEF, HFrEF, 1 year after left ventricular assist device implantation (LVAD), 1 year after heart transplantation (HTx), and healthy controls. All groups individually and overall versus control $P < 0.001$.




## Association with outcome

Median follow-up time in patients with HFpEF and in patients with HFrEF was 522 days [238;1089] and 204 days [56;415], respectively. No patient was lost to follow-up. In HFpEF, the composite endpoint of all-cause death and HF hospitalization occurred in 36 patients, whereof six were deaths. In HFrEF, the composite endpoint of all-cause death, LVAD implantation, or HTx occurred in 56 patients out of which 28 were deaths.

In HFpEF, concentration of NRG1-β was not associated with the composite endpoint (hazard ratio (HR) per log increase 0.75 [95% confidence interval (CI) 0.55–1.04; *P*-value = 0.083]; *Figure 3A*). When stratified by ischaemia, there was an association with outcome (log-rank $P = 0.020$), revealing that ischaemic HFpEF patients with NRG1-β above median had a worse outcome (*Figure 3C*). The pattern was confirmed in the multivariable model but did not reach statistical significance; ischaemic HFpEF (HR 1.07, 95% CI 0.52–2.17, $P = 0.862$) and non-ischaemic HFpEF (HR 0.71, 95% CI 0.48–1.05, $P = 0.085$; $P_{interaction} = 0.553$).

In HFrEF, NRG1-β was prognostic (*Figure 3B*) also in the multivariable model (HR 1.45 [95% CI 1.04–2.03; *P*-value = 0.029]). The association with outcome persisted when stratified for ischaemia (log-rank $P = 0.003$; *Figure 3D*) After adjustments in the multivariable model; in ischaemic HFrEF (HR 1.17, 95% CI 0.68–2.04, $P = 0.569$) and in non-ischaemic HFrEF (HR 1.48, 95% CI 0.93–2.36, $P = 0.098$).

In addition, we analysed outcome stratified by above or below median NRG1-β and NT-proBNP. In HFpEF, worst outcome was in patients below median NRG1-β and above NT-proBNP ($P = 0.049$), while in patients with HFrEF, the strata above median NRG1-β and above median NT-proBNP had the poorest prognosis ($P < 0.001$; Supporting Information, *Figure S1*).

# Discussion

Neuregulin1-β was reduced in both HFpEF and, even more, in HFrEF compared with healthy controls. In HFrEF, concentrations were further decreased after LVAD and HTx. In HFrEF and ischaemic HFpEF, higher levels of NRG1-β were associated with worse outcomes. In contrast, in non-ischaemic HFpEF, it was reversed, and NRG1-β appeared to be potentially protective with higher concentrations tending to be associated with better outcomes. The opposing relationship of NRG1-β with outcomes raises the possibility of a compensatory increase of cardioprotective NRG1-β from the endothelial microvasculature exposed to oxidative stress in non-ischaemic HFpEF, but this compensatory effect is overwhelmed in the presence of ischaemia in HFrEF and ischaemic HFpEF.

## Neuregulin1-β concentrations

We confirm previous findings in HFrEF demonstrating an association between higher concentrations of circulating NRG1-β and HF disease severity.[8,9] In addition, we demonstrate lower concentrations after LVAD and HTx. NRG1, like BNP, has been reported to be cardioprotective and part of the adaptive physiologic response in HF, that is, NRG1, like BNP, is a risk marker for more severe HFrEF and worse outcomes but not a risk factor that causes worse outcomes.[10] The most elevated concentrations of NRG1-β were found in healthy controls. This may be explained by a slightly lower age and absence of inflammation, contributing to a more preserved endothelial function.

We found lower concentrations of circulating NRG1-β in HFrEF compared with HFpEF and controls which is counterintuitive as the neurohormonal activation is greater in HFrEF. Our patients with HFrEF all had severe HF referred for advanced interventions such as LVAD or HTx reflecting significant pump failure in later stages of HF. Animal models suggest that concentric left ventricular hypertrophy and mechanical wall strain initially increase NRG1 mRNA expression.[11] At the same time, the ERBB2 and ERBB4 receptors are downregulated.[7] As terminal HF approaches and pump failure occurs, NRG1 declines potentially due to increasing circulating levels of angiotensin II and epinephrine.[11] Although speculative, the lower NRG1-β concentrations in our patients with HFrEF after intervention could be mediated by a declining neurohormonal activation accompanied by upregulation of the ERBB2 and ERBB4 receptors.[7,12]

**Table 2** Correlates of NRG1-β in the 86 patients with HFpEF and the 86 patients with HFrEF from regression analyses.

| Variable | HFpEF | | | HFrEF | | |
|---|---|---|---|---|---|---|
| | β-coefficient | *P*-value | | β-coefficient | *P*-value | |
| Patient history | | | | | | |
| Age; median (Q1;Q3) | −0.007 | 0.950 | | 0.05 | 0.659 | |
| Gender (men/women) | 1.00 | 0.65–1.54 | 0.987 | 1.02 | 0.54–1.93 | 0.956 |
| Medical history | | | | | | |
| COPD | 0.73 | 0.45–1.20 | 0.219 | 0.87 | 0.41–1.84 | 0.720 |
| T2DM | 0.78 | 0.50–1.21 | 0.269 | 0.76 | 0.45–1.29 | 0.302 |
| Cancer | 0.72 | 0.43–1.19 | 0.200 | 0.77 | 0.33–1.77 | 0.533 |
| Hypertension | 1.24 | 0.74–2.08 | 0.401 | — | — | |
| Atrial fibrillation/flutter | 1.36 | 0.87–2.11 | 0.174 | 1.04 | 0.65–1.68 | 0.870 |
| Coronary artery disease (in ref ischaemic aetiology) | 0.72 | 0.46–1.12 | 0.140 | 0.76 | 0.46–1.24 | 0.265 |
| Measurements | | | | | | |
| Weight (kg) | −0.10 | 0.388 | | −0.03 | 0.742 | |
| BMI (kg/m$^2$) | −0.12 | 0.304 | | −0.03 | 0.815 | |
| Systolic blood pressure (mmHg) | 0.01 | 0.919 | | −0.06 | 0.627 | |
| Diastolic blood pressure (mmHg) | 0.02 | 0.855 | | −0.09 | 0.455 | |
| Heart rate (b.p.m.) | 0.15 | 0.191 | | 0.07 | 0.548 | |
| Treatment | | | | | | |
| ARB | 0.69 | 0.44–1.08 | 0.104 | 31 | (36) | |
| ACE inhibitor | 1.26 | 0.81–1.95 | 0.309 | 51 | 59 | |
| Beta-blocker | 1.05 | 0.62–1.79 | 0.844 | 85 | (99) | |
| Thiazide diuretics | | | | | | |
| Potassium sparing diuretics | 0.94 | 0.56–1.58 | 0.808 | 58 | (67) | |
| Loop diuretics | 1.11 | 0.70–1.76 | 0.652 | 75 | (87) | |
| Calcium channel blocker | 1.06 | 0.67–1.69 | 0.805 | 4 | (5) | |
| Anticoagulants | 1.08 | 0.70–1.67 | 0.717 | 53 | (62) | |
| Antiplatelet | 1.00 | 0.63–1.56 | 0.961 | 22 | (26) | |
| Statins | 0.71 | 0.46–1.12 | 0.140 | 38 | (44) | |
| ECHO parameters | | | | | | |
| LVEF (%) | 0.08 | 0.509 | | −0.04 | 0.733 | |
| LVEDd (mm) | −0.05 | 0.717 | | −0.02 | 0.894 | |
| LAVI (mL/m$^2$) | −0.03 | 0.896 | | | | |
| Left ventricular mass index (g/m$^2$) | | | | | | |
| Men | 0.11 | 0.575 | | | | |
| Women | −0.28 | 0.324 | | | | |
| E/e' ratio | 0.11 | 0.418 | | | | |
| Biochemistry | | | | | | |
| NT-proBNP (ng/L) | 0.07 | 0.545 | | 0.09 | 0.434 | |
| MR-proANP | 0.07 | 0.569 | | 0.20 | 0.171 | |
| MR-proADM | −0.02 | 0.868 | | 0.29 | 0.051 | |
| Hb (g/dL) | **0.25** | **0.029** | | −0.04 | 0.757 | |
| Creatinine (μmol/L) | −0.02 | 0.867 | | 0.08 | 0.460 | |
| eGFR (MDRD) (mL/min/1.73 m$^2$) | 0.02 | 0.887 | | −0.09 | 0.442 | |
| Copeptin | −0.05 | 0.685 | | 0.15 | 0.324 | |
| Sodium | 0.12 | 0.318 | | −0.23 | 0.039 | |
| Potassium | 0.03 | 0.818 | | −0.024 | 0.833 | |
| Glucose fasting (mmol/L) | −0.19 | 0.106 | | −0.12 | 0.344 | |
| Insulin (μU/mL) | −0.14 | 0.248 | | −0.06 | 0.646 | |
| HOMA-IR | −0.19 | 0.103 | | −0.08 | 0.541 | |
| Adiponectine (mg/L) | 0.13 | 0.265 | | **0.33** | **0.004** | |
| Leptin (ng/L) | −0.10 | 0.395 | | −0.04 | 0.741 | |
| IGF1 (mikrog/L) | 0.09 | 0.446 | | −0.07 | 0.543 | |
| SD score IGF1 | 0.05 | 0.677 | | −0.08 | 0.642 | |
| IGFBP1 (mikrog/L) | −0.04 | 0.729 | | **0.37** | **0.001** | |
| IGFBP7 (μg/L) | −0.06 | 0.608 | | **0.31** | **0.008** | |
| sST2 (g/L) | −0.13 | 0.277 | | **0.43** | **<0.0001** | |
| Galectin-3 (g/L) | −0.04 | 0.752 | | 0.17 | 0.139 | |

ACE, angiotensin converting enzyme; ARB, angiotensin II receptor blocker; BMI, body mass index; COPD, chronic obstructive pulmonary disease; eGFR, estimated glomerular filtration rate; Hb, haemoglobin; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; HOMA-IR, homeostatic model assessment of insulin resistance; IGF1, insulin-like growth factor 1; IVRT, isovolumic relaxation time; LAVI, left atrial volume index; LVEDd, left ventricular end diastolic diameter; LVEF, left ventricular ejection fraction; MDRD, Modification of Diet in Renal Disease; MR-proADM, mid-regional pro-adrenomedullin; MR-proANP, mid-regional pro atrial natriuretic peptide; NT-proBNP, N-terminal pro-brain natriuretic peptide; NRG1-β, neuregulin1-β; SD, standard deviation; T2DM, type 2 diabetes mellitus.


DOI: 10.1002/ehf2.12615

**Figure 2** Association between log NRG1-β concentrations and log NT-proBNP and interaction of ischaemia (A) in HFpEF and (B) in HFrEF. Curves depict, with 95% confidence interval, presence of ischaemia in red and no ischaemia present in blue.

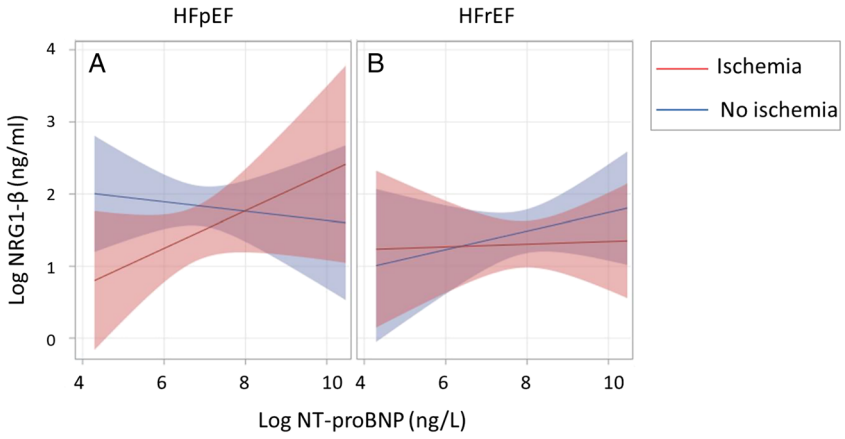
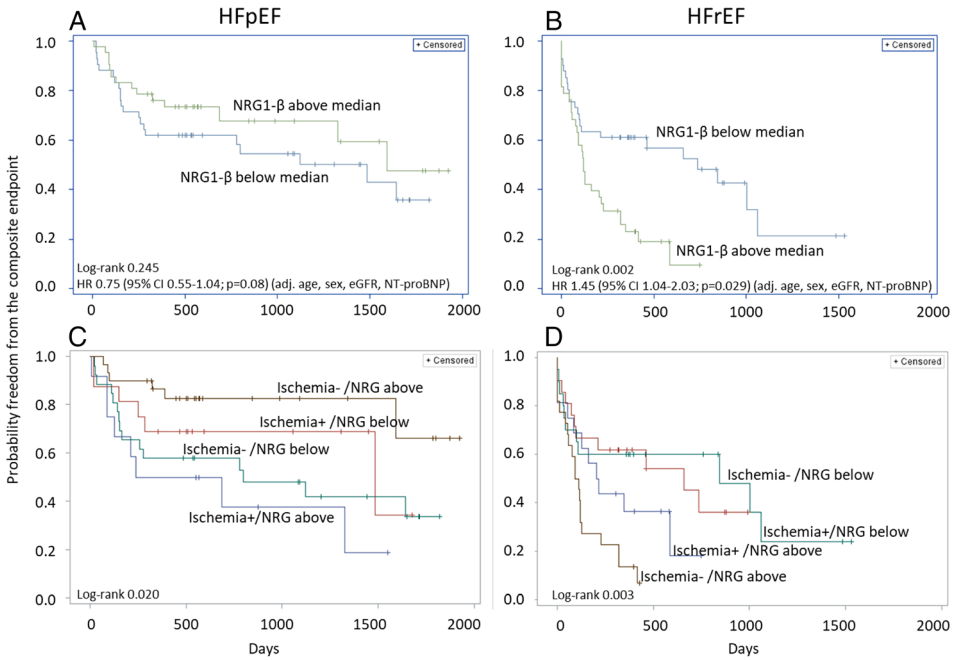



**Figure 3** (A–D) Kaplan–Meier analyses displaying increased survival free of HF hospitalization by median NRG1-β (A) in HFpEF displaying improved prognosis above median NRG1-β; (B) in HFrEF displaying worse prognosis above median NRG1-β; (C) in HFpEF displaying worse prognosis in the presence of ischaemia above median NRG1-β but still improved prognosis in the absence of ischaemia; and (D) in HFrEF displaying worse prognosis above median NRG1-β regardless of the presence of ischaemia.




## Prognostic implications of neuregulin1-β

In concordance with previous findings, we found higher NRG1-β to be associated with increased mortality in HFrEF.[8] In addition, Ky and colleagues suggest NRG1-β as a prognostic predictor in ischaemic but not in non-ischaemic HFrEF. In our study, it appeared prognostic in HFrEF regardless of the presence of ischaemia defined as ischaemic HF aetiology. We add new information on the role of NRG1-β in ischaemic versus non-ischaemic HFpEF. Patients with HFpEF with ischaemia defined as the presence of CAD and a high NRG1-β had the worst outcomes, whereas patients with HFpEF without CAD and high NRG1-β had the best outcomes.

In support of the association between outcome and NRG1-β in ischaemia, it has been shown that NRG/erbB signalling is activated in the setting of ischaemia-reperfusion injury,[13] and

NRG1-β concentrations are initially elevated in patients with stress-induced ischaemia, but as HFrEF progresses, the endothelium is unable to release NRG1-β and concentrations deteriorate. Both NRG1-β mRNA expression and ERBB2 and ERBB4 receptors are downregulated in hypoxic versus normoxic areas,[14] and NRG1 is inversely correlated with angiographic severity.[15]

This raises the possibility, which needs further studies and confirmation, that NRG1-β has diverging roles in the presence and evolvement of ischaemia in HFpEF. In non-ischaemia, declining NRG1-β concentrations may rather reflect the disease-driving oxidative stress contributing to the microvascular endothelial dysfunction that is hypothesized to drive the HFpEF syndrome.[3]

Endothelial dysfunction, highly prevalent in HF regardless of LVEF, is associated with cardiovascular death and HF hospitalization. In the myocardium, NRG1 is primarily produced by the microvascular endothelium in response to mechanical stretch, oxidative stress, and hypoxia.[5] NRG1/ErbB signalling and NRG1 are suggested to increase the number of microvessels in post-ischaemic animal models.[16] Further, demonstrating the link between endothelial dysfunction and oxidative stress, we found that NRG1-β correlated with the oxidative stress marker IGFBP-1 in our patients with HFrEF.

Even if coronary microvascular inflammation and dysfunction occur in both HFrEF and HFpEF, it is suggested to have a more prominent role in the pathophysiology and disease development in HFpEF.[3] Supporting this hypothesis, we recently showed that coronary microvascular dysfunction as defined by reduced coronary flow reserve was present in 75% of patients with HFpEF and also correlated with peripheral endothelial dysfunction.[17]

Neuregulin1 is interesting in this aspect. It may act on both the ventricular and cardiomyocyte levels attenuating endothelial dysfunction and collagen synthesis thus potentially improving passive ventricular stiffness.[18–20] NRG1 has an impact on endothelial dysfunction reducing pro-inflammatory cytokines affecting the pro-fibrotic transforming growth factor beta 1 signalling pathway through ErbB4 receptor activation.[19] It also acts on the cardiomyocyte calcium metabolism, at the level of the sarcoplasmatic reticulum through phosphorylation of phospholamban and correct the altered titin phosphorylation.[21,22]

### Limitations

There are limitations in this relatively small single centre cohort study. Measured plasma NRG1-β reflects the circulation of NRG1 but not necessarily biologically active peptide or receptor activation. The definition of the composite outcome differed between the groups. In HFrEF, a surrogate endpoint reflecting clinical deterioration, that is, HTx or LVAD, was included because these events reduce competing risk. In HFpEF, HF hospitalization was a part of the composite endpoint. We selected these most clinically relevant composite outcomes because we did not compare outcomes in HFrEF versus HFpEF, but we compared associations between NRG1-β and outcomes in HFrEF versus in HFpEF separately. The small sample size may hamper the results and does not allow extensively adjusted survival analyses, but we have adjusted for a few important variables such as renal function, NT-proBNP, age, and sex. Further, we have measured circulating NRG1-β that does not necessarily reflect processes in the myocardium.

## Conclusions

Neuregulin1-β was reduced in both HFpEF and, even more, in HFrEF compared with healthy controls. In HFrEF, concentrations were further decreased after LVAD and HTx. In HFrEF and ischaemic HFpEF, higher levels of NRG1-β were associated with worse outcomes. In contrast, in non-ischaemic HFpEF, it was reversed, and NRG1-β appeared to be potentially protective with higher concentrations tending to be associated with better outcomes. The opposing relationship of NRG1-β with outcomes raises the possibility of compensatory increases of cardioprotective NRG1-β from the endothelial microvasculature exposed to oxidative stress in non-ischaemic HFpEF, but this compensatory effect is overwhelmed in the presence of HFrEF and ischaemic HFpEF.

## Conflict of interest

There are no conflicts of interest related to this study. However, to the extent that findings in KaRen and MetAnEnd may affect the use of heart failure drugs or devices, we disclose the following. C.H. received consulting fees from Novartis and Roche and speaker honoraria from MSD; L.H.L. received research grants from AstraZeneca, Relypsa, Novartis, Boerhinger Ingelheim, and Boston Scientific and consulting or speaker honoraria from Novartis, Vifor, Bayer, Sanofi, Fresenius, and Merck; C.L. received research grants, speaker honoraria, and consulting fees from Medtronic and speaker honoraria and consulting fees from St. Jude Medical; E.D. received speaker honoraria and consulting fees from Novartis and AstraZeneca; J.C.D. received research grants, speaker honoraria, and consulting fees from Medtronic and St. Jude Medical; C.S.P.L. was supported by a Clinician Scientist Award from the National Medical Research Council of Singapore; has received research support from Boston Scientific, Bayer, Roche Diagnostics, AstraZeneca, Medtronic, and Vifor Pharma; has served as consultant or on the Advisory Board/Steering Committee/Executive Committee for Boston Scientific, Bayer, Roche Diagnostics, AstraZeneca, Medtronic,

Vifor Pharma, Novartis, Amgen, Merck, Janssen Research & Development LLC, Menarini, Boehringer Ingelheim, Novo Nordisk, Abbott Diagnostics, Corvia, Stealth BioTherapeutics, JanaCare, Biofourmis, Darma, Applied Therapeutics, WebMD Global LLC, and Radcliffe Group Ltd.

## Funding

None of the funding sources had any role in the design and conduct of the study and in the collection, management, analysis, or interpretation of the data. The work was supported by grants from Center for Gender Medicine Karolinska Institutet, Stockholm Sweden (to C.H. and A.M.B.), and The Prospective KaRen study was supported in part by grants from Fédération Française de Cardiologie/Société Française de Cardiologie, France and Medtronic Bakken Research Center, Maastricht, The Netherlands. L.H.L. was supported by the Swedish Research Council (grant 2013-23897-104604-23), Swedish Heart Lung Foundation (grants 20080409, 20100419), and the Stockholm County Council (grants 00556-2009, 20110120). C.L. was supported by the Swedish Heart Lung Foundation (grants 20080498, 20110406] and the Stockholm County Council (grants 20090376, 20110610).

## Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Figure S1.**Survival free of HF hospitalization above and below median of NRG and NT-proBNP in HFpEF and HFrEF.

## References

1. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JGF, Coats AJS, Falk V, González-Juanatey JR, Harjola V-P, Jankowska EA, Jessup M, Linde C, Nihoyannopoulos P, Parissis JT, Pieske B, Riley JP, Rosano GMC, Ruilope LM, Ruschitzka F, Rutten FH, van der Meer P. 2016 ESC guidelines for the diagnosis and treatment of acute and chronic heart failure. *Eur J Heart Fail* 2016; **18**: 891–975.
2. Marti CN, Gheorghiade M, Kalogeropoulos AP, Georgiopoulou VV, Quyyumi AA, Butler J. Endothelial dysfunction, arterial stiffness, and heart failure. *J Am Coll Cardiol* 2012; **60**: 1455–1469.
3. Paulus WJ, Tschope C. A novel paradigm for heart failure with preserved ejection fraction: comorbidities drive myocardial dysfunction and remodeling through coronary microvascular endothelial inflammation. *J Am Coll Cardiol* [Review] 2013; **62**: 263–271.
4. Timolati F, Ott D, Pentassuglia L, Giraud MN, Perriard JC, Suter TM, Zuppinger C. Neuregulin-1 beta attenuates doxorubicin-induced alterations of excitation-contraction coupling and reduces oxidative stress in adult rat cardiomyocytes. *J Mol Cell Cardiol* 2006; **41**: 845–854.
5. Hedhli N, Huang Q, Kalinowski A, Palmeri M, Hu X, Russell RR, Russell KS. Endothelium-derived neuregulin protects the heart against ischemic injury. *Circulation* 2011; **123**: 2254–2262.
6. Brero A, Ramella R, Fitou A, Dati C, Alloatti G, Gallo MP, Levi R. Neuregulin-1β1 rapidly modulates nitric oxide synthesis and calcium handling in rat cardiomyocytes. *Cardiovasc Res* 2010; **88**: 443–452.
7. Rohrbach S, Niemann B, Silber RE, Holtz J. Neuregulin receptors erbB2 and erbB4 in failing human myocardium—depressed expression and attenuated activation. *Basic Res Cardiol* [Research Support, Non-U.S. Gov't] 2005; **100**: 240–249.
8. Ky B, Kimmel SE, Safa RN, Putt ME, Sweitzer NK, Fang JC, Sawyer DB, Cappola TP. Neuregulin-1β is associated with disease severity and adverse outcomes in chronic heart failure. *Circulation* 2009; **120**: 310–317.
9. Miao J, Huang S, Su YR, Lenneman CA, Wright M, Harrell FE Jr, Sawyer DB, Lenihan DJ. Effects of endogenous serum neuregulin-1β on morbidity and mortality in patients with heart failure and left ventricular systolic dysfunction. *Biomarkers* 2018; **23**: 704–708.
10. Rupert CE, Coulombe KL. The roles of neuregulin-1 in cardiac development, homeostasis, and disease. *Biomark Insights* 2015; **10**: 1–9.
11. Lemmens K, Doggen K, De Keulenaer GW. Role of neuregulin-1/ErbB signaling in cardiovascular physiology and disease: implications for therapy of heart failure. *Circulation* 2007; **116**: 954–960.
12. Uray IP, Connelly JH, Thomazy V, Shipley GL, Vaughn WK, Frazier OH, Taegtmeyer H, Davies PJ. Left ventricular unloading alters receptor tyrosine kinase expression in the failing human heart. *J Heart Lung Transplant* [Research Support, U.S. Gov't, P.H.S.] 2002; **21**: 771–782.
13. Kuramochi Y, Cote GM, Guo X, Lebrasseur NK, Cui L, Liao R, Sawyer DB. Cardiac endothelial cells regulate reactive oxygen species-induced cardiomyocyte apoptosis through neuregulin-1β/erbB4 signaling. *J Biol Chem* [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.] 2004; **279**: 51141–51147.
14. Munk M, Memon AA, Goetze JP, Nielsen LB, Nexo E, Sorensen BS. Hypoxia changes the expression of the epidermal growth factor (EGF) system in human hearts and cultured cardiomyocytes. *PLoS ONE* 2012; **7**: e40243.
15. Geisberg CA, Wang G, Safa RN, Smith HM, Anderson B, Peng XY, Veerkamp B, Zhao DX, Blakemore D, Yu C, Sawyer DB. Circulating neuregulin-1β levels vary according to the angiographic severity of coronary artery disease and ischemia. *Coron Artery Dis* [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't] 2011; **22**: 577–582.
16. Wu C, Gui C, Li L, Pang Y, Tang Z, Wei J. Expression and secretion of neuregulin-1 in cardiac microvascular endothelial cells treated with angiogenic factors. *Exp Ther Med* 2018; **15**: 3577–3581.
17. Shah SJ, Lam CSP, Svedlund S, Saraste A, Hage C, Tan RS, Beussink-Nelson L, Ljung Faxen U, Fermer ML, Broberg MA, Gan LM, Lund LH. Prevalence and correlates of coronary microvascular dysfunction in heart failure with preserved ejection fraction: PROMIS-HFpEF. *Eur Heart J* 2018; **39**: 3439–3450.

18. Mendes-Ferreira P, Maia-Rocha C, Adao R, Mendes MJ, Santos-Ribeiro D, Alves BS, Cerqueira RJ, Castro-Chaves P, Lourenco AP, De Keulenaer GW, Leite-Moreira AF, Bras-Silva C. Neuregulin-1 improves right ventricular function and attenuates experimental pulmonary arterial hypertension. *Cardiovasc Res* 2016; **109**: 44–54.
19. Vermeulen Z, Hervent AS, Dugaucquier L, Vandekerckhove L, Rombouts M, Beyens M, Schrijvers DM, De Meyer GRY, Maudsley S, De Keulenaer GW, Segers VFM. Inhibitory actions of the NRG-1/ErbB4 pathway in macrophages during tissue fibrosis in the heart, skin, and lung. *Am J Physiol Heart Circ Physiol* 2017; **313**: H934–H945.
20. Galindo CL, Kasasbeh E, Murphy A, Ryzhov S, Lenihan S, Ahmad FA, Williams P, Nunnally A, Adcock J, Song Y, Harrell FE, Tran TL, Parry TJ, Iaci J, Ganguly A, Feoktistov I, Stephenson MK, Caggiano AO, Sawyer DB, Cleator JH. Anti-remodeling and anti-fibrotic effects of the neuregulin-1β glial growth factor 2 in a large animal model of heart failure. *J Am Heart Assoc* 2014; **3**: e000773.
21. Adao R, Mendes-Ferreira P, Maia-Rocha C, Santos-Ribeiro D, Rodrigues PG, Vidal-Meireles A, Monteiro-Pinto C, Pimentel LD, Falcao-Pires I, De Keulenaer GW, Leite-Moreira AF, Bras-Silva C. Neuregulin-1 attenuates right ventricular diastolic stiffness in experimental pulmonary hypertension. *Clin Exp Pharmacol Physiol* 2018; **19**: 1440–1681.
22. Hopf A-E, Andresen C, Kötter S, Isić M, Ulrich K, Sahin S, Bongardt S, Röll W, Drove F, Scheerer N, Vandekerckhove L, De Keulenaer GW, Hamdani N, Linke WA, Krüger M. Diabetes-induced cardiomyocyte passive stiffening is caused by impaired insulin-dependent titin modification and can be modulated by neuregulin-1. *Circ Res* 2018; **123**: 342–355.